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Structural Basis of End-Stage Failure in Ischemic Cardiomyopathy in Humans

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Background Ischemic cardiomyopathy is characterized by myocyte loss, reactive cellular hypertrophy, and ventricular scarring. However, the relative contribution of these tissue and cellular processes to late failure remains to be determined.

Methods and Results Ten hearts were obtained from individuals undergoing cardiac transplantation as a result of chronic coronary artery disease in its terminal stage. An identical number of control hearts were collected at autopsy from patients who died from causes other than cardiovascular disease, and morphometric methodologies were applied to the analysis of the left and right ventricular myocardium. Left ventricular hypertrophy evaluated as a change in organ weight, aggregate myocyte mass, and myocyte cell volume per nucleus showed increases of 85%, 47%, and 103%, respectively. Corresponding increases in the right ventricle were 75%, 74%, and 112%. Myocyte loss, which accounted for 28% and 30% in the left and right ventricles, was responsible for the difference in the assessment of myocyte hypertrophy at the ventricular, tissue, and cellular levels. Left ventricular muscle cell hyper-
trophy was accomplished through a 16% and 51% increase in myocyte diameter and length, whereas right ventricular myocyte hypertrophy was the consequence of a 13% and 67% increase in these linear dimensions, respectively. Moreover, a 36% reduction in the number of myocytes included in the thickness of the left ventricular wall was found. Collagen accumulation in the form of segmental, replacement, and interstitial fibrosis comprised an average 28% and 13% of the left and right ventricular myocardia, respectively. The combination of cell loss and myocardial fibrosis, myocyte lengthening, and mural slippage of cells resulted in 4.6-fold expansion of left ventricular cavitary volume and a 56% reduction in the ventricular mass-to-chamber volume ratio.

Conclusions These results are consistent with the contention that both myocyte and collagen compartments participate in the development of decompensated eccentric ventricular hypertrophy in the cardiomyopathic heart of ischemic origin. (*Circulation*. 1994;89:151-163.)

Key Words • hypertrophy • collagen • dilation

Ischemic cardiomyopathy is an anatomic condition initiated by primary events in the coronary circulation that lead to myocyte loss, scarring, and ventricular failure. Cell loss occurs as a result of narrowing and/or occlusion of coronary arteries by atherosclerosis, spasm of major or intramural arterial branches of the coronary vasculature, or alterations of the microcirculation, which, alone or in combination, produce varying degrees of ischemia and tissue injury.[1-6] The clinical spectrum ranges from acute myocardial infarction to chronic ischemic cardiomyopathy. The latter case may take the form of a dilated ischemic myopathy characterized by multiple focal sites of myocardial damage in the ventricular wall.[7] In the most common manifestation, in which scattered foci of replacement fibrosis are found in conjunction with a healed infarct, the question remains concerning the pathophysiological significance of these two types of myocardial lesions. Specifically, it has not been established whether the segmental loss of myocar-
dium associated with coronary artery occlusion and infarction is the principal determinant of myocyte cell loss, wall thinning, ventricular dilation, and worsening of the hemodynamic performance with time or whether the multiple isolated sites of tissue injury in the noninfarcted portions of the wall are major factors in the progression of the cardiac myopathy. In addition, both forms of ischemic damage reduce the amount of functioning myocardium, increasing the load on the surviving region of the ventricle, which undergoes compensatory reactive hypertrophy.[6-9] This growth process may expand the length of the unaffected myocytes more than myocyte diameter, contributing to chamber dilation and relative thinning of the wall.[10,11] Ventricular dilation has repeatedly been demonstrated to constitute an unfavorable outcome of the ischemic cardiomyopathic heart acutely and chronically.[12-16] Moreover, this anatomic factor has been shown to limit survival in both humans and animal models.[9] Therefore, the present investigation was undertaken to analyze the quantitative structural properties of hearts removed from patients undergoing cardiac transplantation as a result of chronic ischemic heart disease and refractory congestive heart failure. Hearts collected at autopsy from individuals who died from causes other than cardiovascular disease were used as controls.

Methods

Patient Populati n

Ten hearts were obtained from male patients who underwent cardiac transplantation at the University Hospital of Udine

Medical School from June 1989 t May 1992. These patients had a history of myocardial infarction and severe coronary artery disease. Intractable congestive heart failure was the cause of cardiac transplantation. An additional group of 10 hearts was collected at autopsy, within 24 hours after death, and assumed to represent normal hearts. According to preautopsy criteria, autopsy criteria, and histological criteria that have been described in detail previously,[17] these hearts were used as controls. In 7 of the 10 patients whose hearts were used as controls, death was sudden and provoked by a traumatic injury. In the remaining 3 cases, cerebral hemorrhage was found in 2 and pulmonary thromboembolism in 1. In these 3 patients, death occurred within 5 days after hospitalization.

Cardiac Weight

After excision of the heart, the great vessels were removed, the atria were dissected free along the atrial ventricular groove, and the coronary arteries were cut perpendicularly to their course for the assessment of the degree of atherosclerosis. The epicardial fat was then carefully removed, the valves were cut free, and the weights of the left ventricle inclusive of the septum and right ventricular free wall were determined. Ventricular mass volume was then computed by dividing ventricular weight by the specific gravity of muscle tissue, 1.06 g/mL.[18] Before sectioning, the major longitudinal intracavitary axis of the left ventricle was determined.[19]

Preparation of Myocardial Specimens

The two ventricles of each heart were sliced into seven sections, approximately 15 mm in thickness, perpendicularly to the major axis of the heart from the apex to the base. These sections were then examined grossly for the assessment of large areas of myocardial fibrosis. The midsection (No. 4) was used to estimate transverse chamber diameter and wall thickness.[17] Subsequently, the two middle slices of the free wall of each ventricle (sections 4 and 5) were cut radially to obtain tissue fragments extending from the endocardial to the epicardial surface. These samples were fixed in 10% buffered formalin and embedded in paraffin. An additional sampling of two tissue blocks from each remaining slice of each ventricle and two from the interventricular septum was obtained and embedded in paraffin for further estimation of the presence of myocardial fibrosis and tissue injury.

Tissue Sampling for the Estimation of Replacement Fibrosis

Quantitative analysis was restricted to the two transverse myocardial slices of both ventricles, which were completely processed for histological examination. These multiple samples were sectioned and stained with hematoxylin and eosin and trichrome. Subsequently, each stained histological section was examined with a microscope equipped with a camera connected to a computerized image-analysis system (IBAS; Zeiss, Oberkochen, Germany). For the evaluation of the volume percent of the myocardium occupied by foci of replacement fibrosis, all sections obtained from the two transverse myocardial slices of each ventricle were evaluated with a ×2.5 objective that defined a final tissue area of 4.4 mm². By this approach, the areas of tissue occupied by these discrete myocardial lesions and normal myocardium were determined to compute their relative volumes in each ventricle.[20]

Tissue Sampling for the Estimation f Myocyte Nuclear Numerical Density and Myocyte Nuclear Length

Six randomly chosen embedded tissue blocks from each ventricle were sectioned at a thickness of 2 to 3 μm and stained with hematoxylin and eosin. These sections were examined with a ×63 objective, which defined a tissue area of 9911 μm². Morphometric sampling consisted of counting the total number of myocyte nuclear profiles, $N(n)$, in a measured area, A, of tissue sections in which cardiac muscle fibers were sectioned transversely. A square tissue area was delineated in the projected field by a grid containing 42 sampling points. One hundred fields were evaluated in each ventricle of each heart t determine the mean number of nuclear profiles per unit area of myocytes, $N(n)_A$. Average nuclear length, D_n, was determined from 50 measurements in each ventricle, which were made at a magnification of ×630 in longitudinally oriented myocytes. Only those nuclei in which the nuclear envelope was sharply defined at both ends and in which clusters of mitochondria were clearly visible in the areas adjacent to the nuclear edges were measured.[21] In addition, myocyte diameter, d, in the region of the nucleus was obtained in these cells.

Tissue Sampling for Estimation of Volume Composition of Myocardium

The same tissue blocks used for the evaluation of nuclear numerical density and length were used here. However, sections were stained with trichrome, and their projected images were examined with the morphometric grid indicated above. In particular, the volume percent of myocytes in the tissue, $V(m)_V$, was obtained by counting the fraction of points overlying the myocyte compartment in each of the 100 fields examined.[20] Similarly, the volume fraction of the nonmyocyte compartment occupied by interstitial fibrosis and vascular framework was estimated from the number of points lying over these two tissue components.

Morphometric Calculations

The data of myocyte nuclear numerical density, $N(n)_A$, nuclear length, D_n, and volume fraction of myocytes in the myocardium were combined to yield the average measurement of the number of myocyte nuclei per unit volume of myocytes, $N(n)_V$, in the ventricle using the following equation[20,21]:

(1) $$N(n)_V = N(n)_A / D_n$$

The aggregate volume of myocytes in the ventricle, $V(m)_T$, was then derived from the ventricular volume measurement, V and $V(m)_V$:

(2) $$V(m)_T = V \times V(m)_V$$

The total number of myocyte nuclei in the ventricle, $N(n)_T$, was computed from $N(n)_V$ and $V(m)_T$:

(3) $$N(n)_T = N(n)_V \times V(m)_T$$

$V(m)_T$ divided by $N(n)_T$ yielded the average myocyte cell volume per nucleus, $V(m)_n$, in the ventricle in each heart:

(4) $$V(m)_n = V(m)_T / N(n)_T$$

Myocyte length per nucleus, $L(m)_n$, was derived from the quotient of myocyte cell volume per nucleus, $V(m)_n$, and myocyte diameter in the region of the nucleus, d:

(5) $$L(m)_n = V(m)_n / d$$

Finally, the estimations of the volume fraction of replacement fibrosis and interstitial fibrosis were combined with the derivation of ventricular volume to yield the aggregate volumes of these component structures in the myocardium.

Statistical Analysis

All morphometric data were collected blindly, and the code was broken at the end of the experiment. Results are presented as mean±SD computed from the average measurements obtained from each heart. Statistical significance for comparison between two measurements was determined using the unpaired two-tailed Student's *t* test. Values of $P<.05$ were considered to be significant.

TABLE 1. Gross Cardiac Characteristics

Characteristic	Controls	Ischemic Cardiomyopathy	Difference, %	P
Age, y	51±6	52±9	2	NS
Body weight, kg	78±11	72±7	−8	NS
Height, cm	173±8	174±9	0	NS
Body surface, m²	1.92±0.15	1.86±0.14	−3	NS
Heart weight, g	217±27	306±45	82	<.001
Left ventricular weight, g	163±23	301±49	85	<.001
Right ventricular weight, g	54±6	95±33	75	<.005
Heart weight/body weight, g/kg	2.78±0.44	5.49±0.703	97	<.0001
Left ventricular weight/body weight, g/kg	2.111±0.359	4.171±0.524	98	<.0001
Right ventricular weight/body weight, g/kg	0.702±0.102	1.355±0.591	93	<.005
Heart weight/body surface, g/m²	113±14	213±25	88	<.0001
Left ventricular weight/body surface, g/m²	85±12	162±21	90	<.0001
Right ventricular weight/body surface, g/m²	28±3	51±21	84	<.005
Thickness of left ventricular wall, mm				
Anterior wall	13.8±1.8	15.5±4.2	13	NS
Lateral wall	14.1±1.9	12.4±3.7	−12	NS
Posterior wall	13.9±1.4	10.7±4.9	−23	NS
Average	13.9±1.6	12.7±3.1	−8	NS
Thickness of right ventricular wall, mm				
Anterior wall	4.91±1.25	6.21±1.78	26	NS
Posterior wall	4.66±0.61	6.76±2.88	45	<.05
Average	4.78±0.78	6.48±1.92	36	<.025

Results are presented as mean±SD.

Results

Clinical Data

The 10 patients affected by ischemic cardiomyopathy had New York Heart Association functional class III or IV. The duration of heart failure was 23±24 months (range, 5 to 84 months). Ejection fraction was 22±8%. Diabetes and pulmonary hypertension singularly or in combination were found in 4 of the 10 patients. Chronic obstructive pulmonary disease and thalassemia minor were seen in 2 of these 4 cases. None of the patients had a history of systemic hypertension, but all suffered from a previous myocardial infarction. The initial myocardial infarction was as remote as 14 years and as recent as 8 months before transplantation, averaging 6±4 years in the entire group. Six patients previously had received single or multiple coronary bypass surgery. Left ventricular aneurysm was found in 4 patients.

Control hearts were obtained from individuals similar in age, body weight, body surface, and height (Table 1) who died not as a result of primary heart disease or as a consequence of major risk factors of coronary artery disease, including hypertension, diabetes, obesity, and/ r severe atherosclerosis. In addition, the autopsy report and the histological examination of all organs excluded diffuse, metastatic malignant neoplasms and chronic inflammatory states. In three of the seven patients in whom death was associated with a traumatic injury (see "Methods"), a complete medical history could not be obtained. Their suitability as contr ls was

based on the absence of a previous hospital record and on the fact that the autopsy failed to reveal underlying pathological processes, with the exception of the damage produced by the acute traumatic injury. A detailed description of the multiple criteria used to characterize a normal individual and a nonpathological heart has recently been provided.[17]

Anatomic Parameters

Table 1 shows the age of the patients and the gross characteristics of the left and right ventricles after careful dissection of the epicardial fat. Ischemic heart disease was associated with an 85% and 75% increase in left and right ventricular weight, respectively. When cardiac weights were normalized by body weight, left and right ventricular weight–to–body weight ratio increased by 2.0-fold and 1.9-fold, respectively. Similar changes were seen with regard to body surface. The thickness of the anterior, lateral, and posterior aspects of the left ventricular wall was comparable in normal and diseased hearts, whereas average right ventricular wall thickness increased 36% with ischemic cardiomyopathy.

Fig 1 illustrates that the transverse chamber diameter of the left ventricle at the equatorial region of the heart increased 99% (P<.0001) with ischemic cardiomyopathy. Because the longitudinal intracavitary axis expanded 15% (P<.05), the calculated chamber volume[22] was found to be nearly 4.6-fold (P<.0001) greater. This magnitude of ventricular dilation provoked a 56% (P<.0001) reduction in the ventricular mass–to–cham-
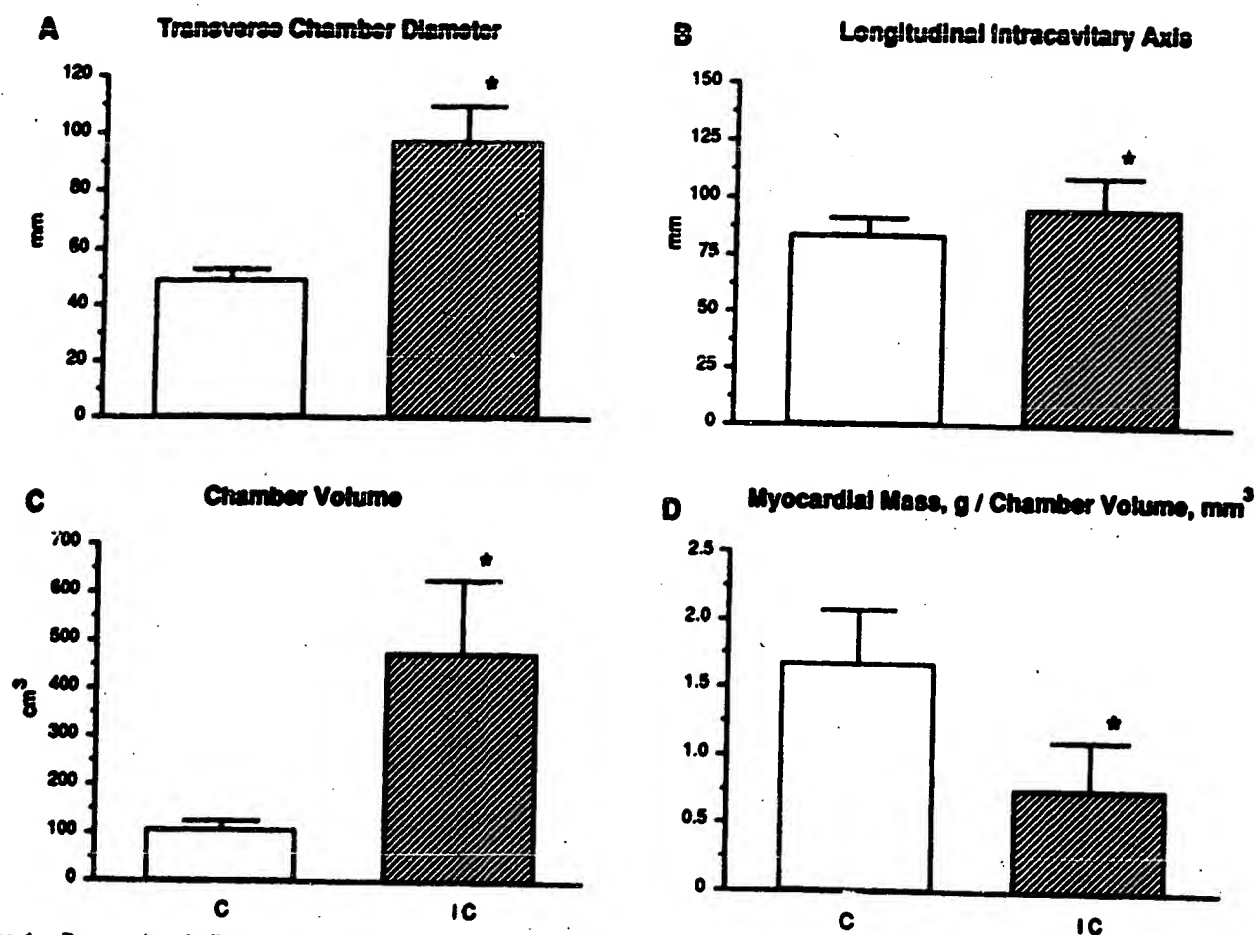
Fig 1. Bar graphs of effects of ischemic cardiomyopathy (IC) on the dimensional properties of the left ventricle. C indicates control hearts. Results are presented as mean±SD. *Indicates a value that is statistically different.

ber volume ratio in the absence of an increase in the average thickness of the wall (Table 1).

In view of the difficulty in the determination of right ventricular chamber volume, right ventricular wall area was calculated as previously performed.[23] The computation of ventricular wall area, which is obtained by dividing wall volume by wall thickness,[23] assumes that the ventricular wall may be treated as a thin sheet. Thus, increases in wall area imply larger chamber volumes. By this approach, it was found that this parameter was 109 ± 18 cm^2 in normal hearts and 149 ± 46 cm^2 in ischemic cardiomyopathy. The 37% difference was found to be statistically significant ($P < .05$). However, such an expansion in wall area was significantly less than the magnitude of hypertrophic growth in the right ventricular wall (Table 1).

In summary, ischemic cardiomyopathy led to cardiac hypertrophy and ventricular dilation. However, the expansion in chamber volume exceeded the growth response of the myocardium in the left ventricle, whereas an opposite effect was found in the right ventricle.

Myocardial Structure

Gross sectioning of the coronary vasculature revealed severe diffuse coronary atherosclerosis involving the three major vessels in all cases. Occlusions of one or two coronary arteries were found in each of the 10 cases. Although histological examination did not show thick-

ening of the wall or other pathological changes of the intramural branches of the coronary arterial tree, the impossibility of fixing the myocardium by perfusion of the coronary vasculature limited the light microscopic analysis of intermediate-sized arteries and arterioles. Thus, an accurate study of the intramyocardial coronary artery branches could not be performed.

Segmental fibrosis, replacement fibrosis, and interstitial fibrosis are used as quantitive definitions. In this investigation, segmental fibrosis defines a healed myocardial infarct that comprises an area of myocardium >1 cm^2, whereas replacement fibrosis describes discrete areas of myocardial scarring developed as a result of focal myocyte cell loss. These sites of myocardial injury vary in size but are <1 cm^2. Finally, interstitial fibrosis corresponds to widening of the interstitial space with collagen accumulation in the absence of apparent myocytolytic necrosis and focal cell death. These three forms of tissue injury have been described previously (for review, see Reference 7) to distinguish the effects of occlusion of a major coronary artery from those involving small branches of the coronary circulation r activation of interstitial fibroblasts, independent of myocyte cell death. Figs 2A, 2B, 3A, and 3B illustrate these different aspects of myocardial damage.

Gross inspection of the slices of the left ventricle inclusive of the interventricular septum and right ventricle of each heart revealed segmental areas of myocardial

FIG 2. Photomicrographs of sections of paraffin-embedded left ventricular tissue showing a healed myocardial infarct with thinning of the wall (A) and multiple sites of replacement fibrosis in the noninfarcted viable left ventricular tissue (B). Trichrome staining. A, ×9. B, ×8. Bar corresponds to 2 mm in A and B.

fibrosis consistent with a previous myocardial infarct. However, these sites of myocardial scarring were restricted to the left ventricle. Some aspects of these areas of damage are illustrated in Fig 2A, which was obtained by low-power light microscopy of large sections of paraffin-embedded material. In addition, tissue sections of the noninfarcted myocardium demonstrated that ischemic cardiomyopathy was accompanied by multiple sites of myocardial injury across the wall of the left and right ventricles (Fig 2B). These lesions were more numerous in the endomyocardium than in the midmyocardium and epimyocardium of both ventricles. Such patchy areas of replacement fibrosis consisted of collagen accumulation surrounding at times small myocyte profiles. Large fibroblasts dispersed within collagen also were present (Fig 3A). Furthermore, the myocardial tissue, free of th se foci of damage, showed diffuse interstitial fibrosis (Fig 3B) that involved the wall of the left and right ventricles. Myocytes were enlarged, although a certain variability in muscle cell size was noted. Similar structural characteristics were seen in the multiple myocardial sam-
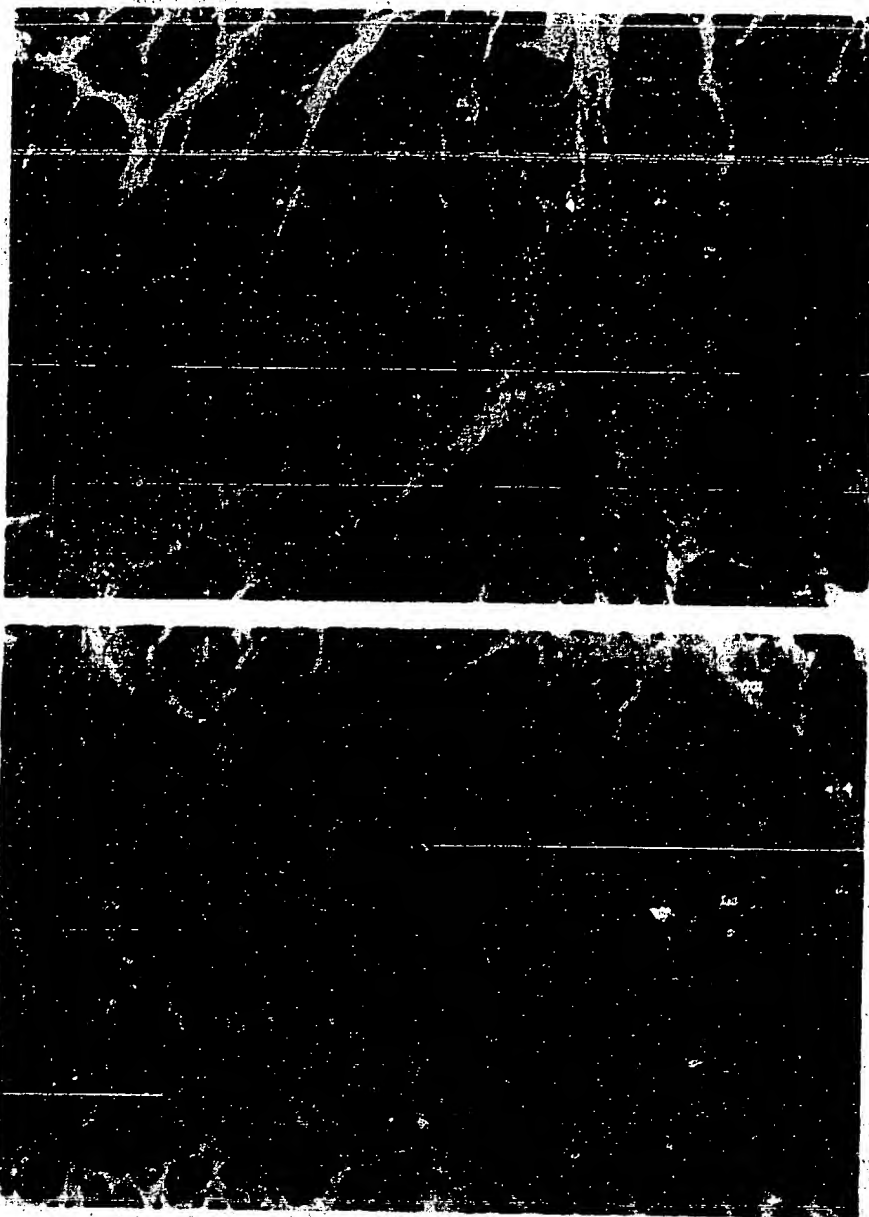
Fig 3. Photomicrographs of left ventricular tissue sections of myocardium remote from the infarcted region showing small areas of replacement fibrosis and bundles of collagen surrounding small clusters of myocytes of variable size (A). Diffuse interstitial fibrosis of the myocardium is also apparent (B). Hematoxylin and eosin staining. A, ×500. B, ×270. Bar corresponds to 25 μm in A and 50 μm in B.

ples included in the analysis of each heart. These morphological findings were observed in all 10 cases examined.

In summary, ischemic cardiomyopathy was characterized by left ventricular myocardial infarction and by multiple foci of replacement fibrosis and diffuse interstitial fibrosis, which affected the noninfarcted myocardium of both ventricles.

Quantitative Analysis of Myocardial Fibrosis

Fig 4A illustrates the contribution of segmental myocardial scarring, replacement fibrosis, and interstitial fibrosis to the accumulation of collagen in the left and right ventricles with ischemic cardiomyopathy. In the left ventricle, the volume percent of myocardial scarring associated with healed infarcts was nearly 9%, whereas replacement fibrosis involved an average 14% of the ventricular wall. In addition, interstitial fibrosis comprised approximately 6% of the nondamaged myocardium. In comparison with normal left ventricles, the relative amounts of

replacement and interstitial fibrosis were increased fourfold ($P<.005$) and twofold ($P<.0001$), respectively. When the three different forms of myocardial fibrosis were combined, 28% of the ventricular wall was found to be represented by fibrotic tissue in ischemic cardiomyopathy. This magnitude of collagen constituted a 4.5-fold ($P<.0001$) increase with respect to control hearts.

Fig 4B documents the regional distribution of replacement fibrosis in the left ventricular wall. This parameter appeared to be higher in the endomyocardium, intermediate in the midmyocardium, and lower in the epimyocardium in both normal and pathological hearts. On the other hand, ischemic cardiomyopathy was characterized by a 3.9-fold ($P<.005$), 4.0-fold ($P<.005$), and 4.5-fold ($P<.005$) increase in replacement fibrosis in the inner, middle, and outer layers of the wall, respectively. In addition, the individual foci of replacement fibrosis were variable in size, ranging from a minimum of 230 μm^2 to a maximum of 80×10^6 μm^2. This latter cross-sectional area was encountered in one patient only once. The second

Myocardial Fibrosis   **Transmural Distribution of Replacement Fibrosis**

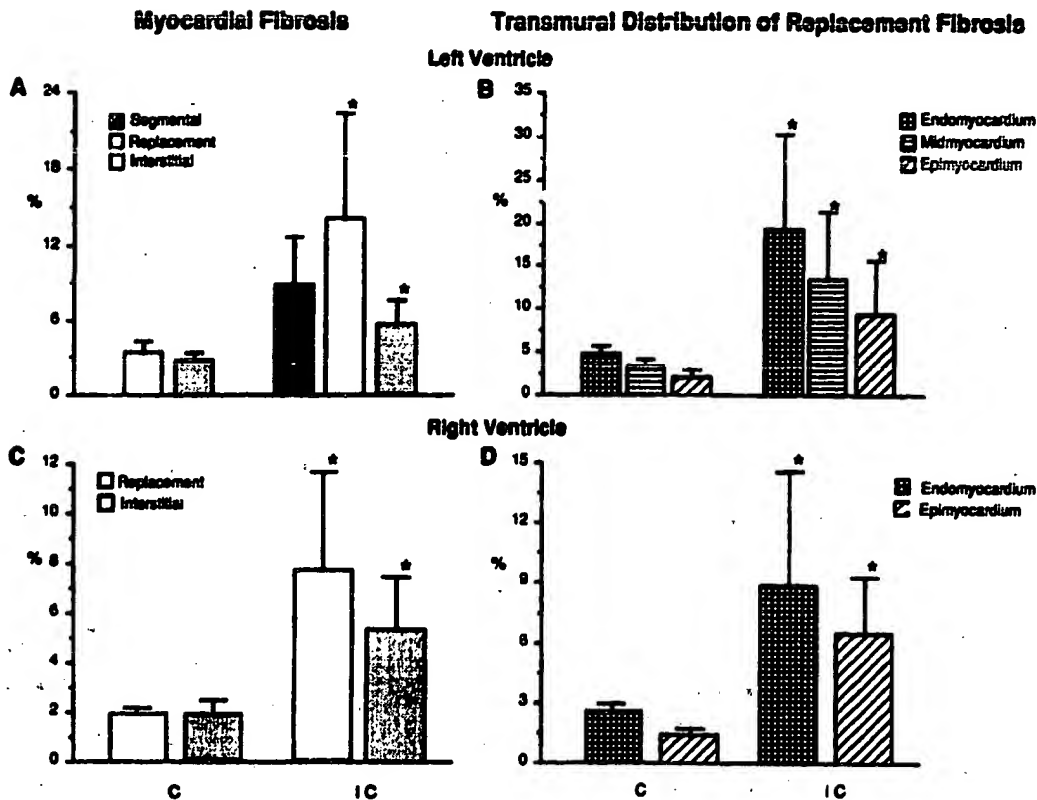**Left Ventricle**

Right Ventricle

FIG 4. Bar graphs of effects of ischemic cardiomyopathy (IC) on the relative amounts of segmental, replacement, and interstitial fibrosis in the myocardium. C indicates control hearts. Results are presented as mean±SD. *Indicates a value that is statistically different.

larger site of replacement fibrosis was $30 \times 10^6 \ \mu m^2$ and the third was $25 \times 10^6 \ \mu m^2$, both of which were seen as isolated examples in two separate cases. Thus, the quantification of replacement fibrosis included foci $<1 \ cm^2$. This dimension was assumed to represent the upper limit for this form of myocardial damage. Such an arbitrary approach implied that areas of myocardial scarring $>1 \ cm^2$ had to be considered as microinfarcts.

Fig 4C shows the volume fraction of replacement and interstitial fibrosis in the right ventricular myocardium. Ischemic cardiomyopathy resulted in 4.0-fold ($P<.001$) and 2.8-fold ($P<.0001$) increases in these two forms of myocardial scarring, which together were found to comprise an average 13% of the ventricular wall. Moreover, the mural distribution of replacement fibrosis (Fig 4D) was similar to that seen in the left ventricle (Fig 4B), because this tissue parameter occupied a larger fraction of the endomyocardium than of the epimyocardium. However, similar increases in the relative volume of replacement fibrosis in the endomyocardium (3.7-fold; $P<.005$) and epimyocardium (4.5-fold; $P<.0001$) were detected with ischemic cardiomyopathy.

In summary, ischemic cardiomyopathy was characterized by segmental myocardial scarring, replacement fibrosis, and interstitial fibrosis that markedly altered the normal structure of the myocardium.

Number of Myocyte Nuclei in the Ventricles

The primary measurements that were used for derivation of the numerical density of myocyte nuclei per unit volume of myocytes consisted of the determination of the number of myocyte nuclei per unit area of myocytes and the evaluation of average nuclear length. The products of

these parameters with the aggregate volume of myocytes in the ventricular myocardium yielded the total number of myocyte nuclei in the ventricles.[21] The results obtained in normal and diseased hearts are shown in Fig 5. In comparison with control hearts, the left ventricle of cardiomyopathic hearts showed a 28% ($P<.01$) loss in the total number of myocyte nuclei. This measurement included the contribution of myocardial infarction. A 30% ($P<.05$) loss in myocyte nuclei was found in the right ventricle. It should be noted that these decreases in the total number of myocyte nuclei in the ventricles correspond to identical changes in myocyte number if the proportion between mononucleated and binucleated cells in the myocardium remains essentially constant.

In summary, ischemic cardiomyopathy led to a significant amount of myocyte loss in the ventricular myocardium.
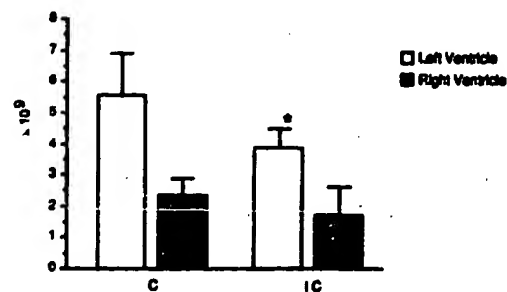

FIG 5. Bar graph of effects of ischemic cardiomyopathy (IC) on the total number of myocyte nuclei in the ventricular wall. C indicates control hearts. Results are presented as mean±SD. *Indicates a value that is statistically different.
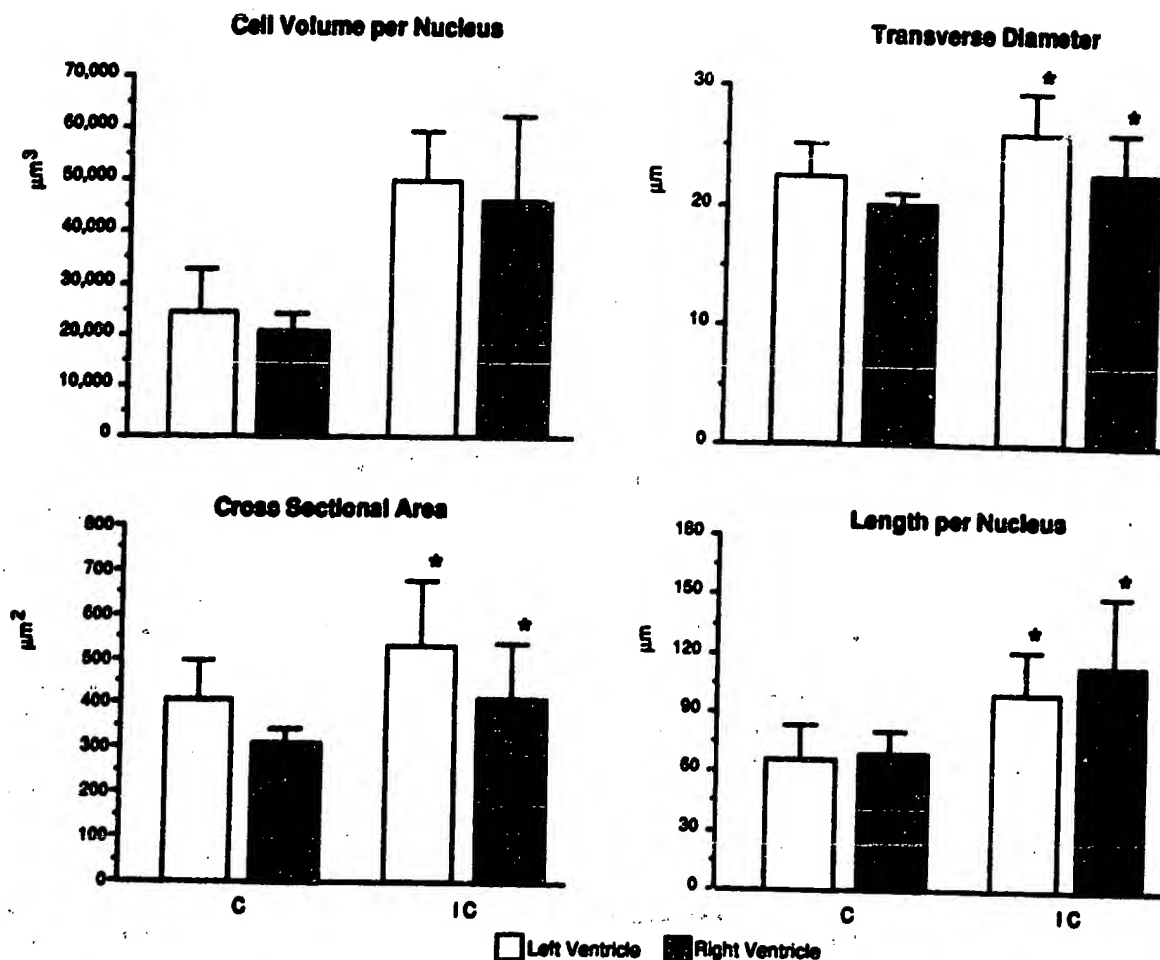
Fig 6. Bar graphs of effects of ischemic cardiomyopathy (IC) on the dimensional properties of left and right ventricular myocytes. C indicates control hearts. Results are presented as mean±SD. *Indicates a value that is statistically different.

Myocyte Cell Volume per Nucleus

Fig 6 shows the measurements of myocyte cell volume per nucleus obtained in the left and right ventricles of control and pathological hearts. In comparison with normal hearts, myocyte cell volume per nucleus with ischemic cardiomyopathy increased 103% ($P<.0001$) and 112% ($P<.0001$) in the left and right ventricles, respectively. Because average myocyte diameter and cross-sectional area expanded by 16% ($P<.05$) and 34% ($P<.05$) in left myocytes, mean myocyte length per nucleus was found to be increased by 51% ($P<.005$). Corresponding increases in myocyte diameter, cross-sectional area, and length per nucleus in the right ventricle were 13% ($P<.05$), 27% ($P<.05$), and 67% ($P<.005$). These cellular parameters were all collected in myocardial tissue remote from the infarcted area.

The availability of wall thickness measurements, volume fraction of myocytes, and myocyte diameter allowed the computation of the average number of myocyte profiles included in the thickness of the left ventricular wall.[20] This analysis revealed that the mural number of myocytes in control and diseased left ventricles was 541±105 and 344±128, respectively. Thus, ischemic cardiomyopathy resulted in a 36% ($P<.005$) reduction in the number of myocytes in the wall. In contrast, the mural number of myocytes remained constant in the right ventricle, from a value of 204±35 in controls to a value of 219±73 in myopathic hearts.

In summary, ischemic cardiomyopathy was characterized by biventricular myocyte hypertrophy in which the increases in cell length exceeded the lateral expansion of myocytes. Moreover, side-to-side slippage of myocytes may have occurred in the left ventricular wall.

Aggregate Volume Changes in the Heart

Fig 7 shows the effects of ischemic cardiomyopathy on the absolute constituent volumes of the left and right ventricular myocardia. These results were obtained by multiplying the volume percent of the different components of the collagen compartment (Fig 4) and volume fraction of myocytes (data not shown) by the overall tissue volume. Tissue volume was obtained from the quotient of the ventricular weight and the specific gravity of muscle.[18] In comparison with control left ventricles, ischemic cardiomyopathy was seen to produce a 47% ($P<.001$) increase in the aggregate volume of myocytes and a 7.4-fold ($P<.001$) and 3.7-fold ($P<.0001$) increase in the total volume of replacement and interstitial fibrosis in the entire left ventricular myocardium. When the amount of collagen associated with the segmental loss of myocardium was combined with the magnitude of collagen coupled with replacement and interstitial fibrosis, the expansion of the whole collagen compartment was found to be 8.3-fold ($P<.0001$). Thus, the increase in connective tissue ex-
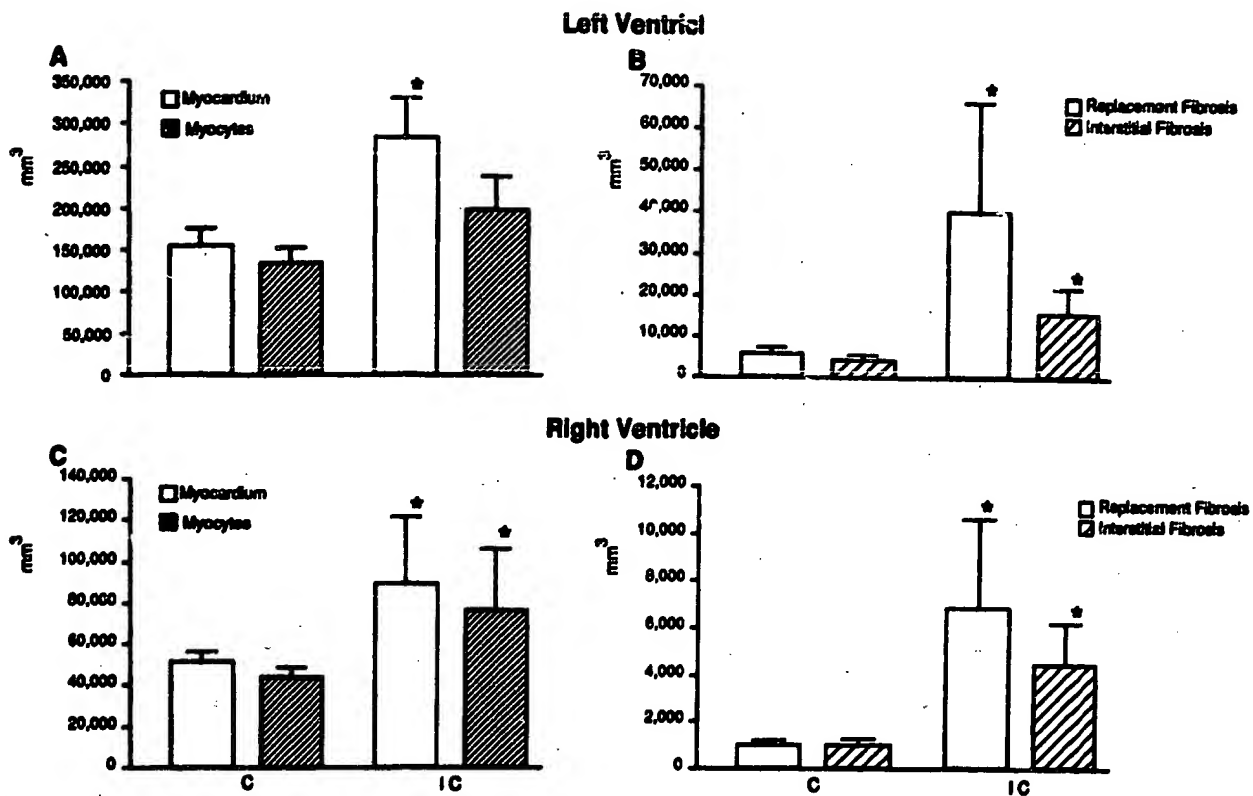
Left Ventricl

Right Ventricle

FIG 7. Bar graphs of effects of ischemic cardiomyopathy (IC) on the absolute amounts of myocytes and replacement and interstitial fibrosis in the left and right ventricles. C indicates control hearts. Results are presented as mean±SD. *indicates a value that is statistically different.

ceeded the increase in left ventricular mass, which, in turn, exceeded the expansion in total myocyte volume.

Figs 7C and 7D illustrate how ischemic cardiomyopathy affected the absolute amounts of myocytes and collagen in the right ventricular wall. The myocyte compartment was seen to be increased by 74% ($P<.01$). In addition, the magnitude of replacement and interstitial fibrosis increased 6.9-fold ($P<.0001$) and 4.6-fold ($P<.0001$). As a whole, connective tissue increased 5.3-fold ($P<.0001$). Thus, the accumulation of collagen in the right ventricular myocardium was greater than the overall increase in right ventricular and myocyte mass.

Table 2 lists the relative and absolute amounts of segmental, replacement, and interstitial fibrosis in the left and right ventricular myocardia of each cardiomyopathic heart. In 6 of the 10 cases, the magnitude of replacement fibrosis in the left ventricle exceeded the extent of scarring associated with myocardial infarction. In 3 instances, segmental fibrosis involved a larger portion of the left ventricle than replacement fibrosis, whereas both forms of scarring were essentially identical in the 1 remaining case. In 8 cases, replacement and interstitial fibrosis combined represented the major source of collagen accumulation in the left ventricle. Finally, the analysis of the seven slices of each left ventricle (see "Methods") demonstrated that a single myocardial infarct was found in eight cases and two infarcts in two cases (hearts 5 and 6 in Table 2). These were healed infarcts and n acute lesi ns were n ted.

In patient 5 (Table 1), there was an infarct comprising 8% of the anterolateral aspect of the left ventricular

free wall and a second infarct involving 5% of the posterior portion of the left ventricle. These areas of segmental fibrosis were associated with severe atherosclerosis and marked reductions in luminal diameter of the left anterior descending and left and right circumflex coronary arteries. Patient 6 (Table 1) had two infarcts affecting 10% and 7% of the interventricular septum and posterior region of the left ventricular wall, respectively. Coronary atherosclerosis with severe narrowing of all major epicardial coronary arteries was present. In the remaining eight cases, the size of each infarct is listed first in Table 2 as segmental fibrosis. It should be emphasized that foci of myocardial scarring <1 cm^2 were not included in this category.

In the right ventricle, segmental fibrosis was absent in all cases, and replacement fibrosis was the predominant form of collagen accumulation in six hearts. Interstitial fibrosis exceeded replacement fibrosis in three instances, whereas similar values in these two aspects of myocardial damage were seen in one case. In comparison with the left ventricle, the volume percent of replacement fibrosis in the corresponding right ventricle was significantly less in 9 of the 10 cardiomyopathic hearts. The relative amount of interstitial fibrosis was more variable in the 2 ventricles. This parameter was higher in the left ventricle in 4 cases, less in 4, and similar in 2. However, in 9 hearts, the volume fraction of replacement and interstitial fibrosis combined was lower in the right ventricle than in the left ventricle.

In summary, ischemic cardiomyopathy was characterized by accumulation of collagen biventricularly, mostly in the form of replacement and interstitial fibrosis.

TABLE 2.   Relative and Absolute Amounts of Segmental, Replacement, and Interstitial Fibrosis in the Left and Right Ventricles of Each Cardiomyopathic Heart

	Heart									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Left ventricle, vol %										
SF	4.28	7.26	9.38	7.95	13.20	16.87	10.73	8.32	4.59	5.02
RF	19.70	25.40	25.90	13.29	12.50	5.62	22.37	5.37	2.79	7.42
IF	7.97	5.21	3.51	7.13	7.07	3.33	5.84	2.68	8.40	4.65
RF+IF	27.67	30.61	29.41	20.42	19.57	8.95	28.21	8.05	11.19	12.07
Aggregate volume, cm³										
SF	15.7	19.9	29.2	17.6	26.6	53.5	29.9	22.1	13.0	16.1
RF	72.3	69.5	80.6	29.3	25.2	17.8	62.2	14.3	7.9	23.8
IF	29.3	14.3	10.9	15.7	14.3	10.6	16.3	7.2	23.8	14.9
RF+IF	101.6	83.8	91.5	45.0	39.5	28.4	78.5	21.5	31.7	38.7
Right ventricle, vol %										
RF	5.37	7.40	8.66	4.78	4.17	11.33	17.30	6.06	3.76	7.60
IF	6.71	7.62	4.49	5.97	2.59	3.16	9.19	2.89	4.97	5.88
RF+IF	12.08	15.02	13.15	10.75	6.76	14.49	26.49	8.95	8.73	13.48
Aggregate volume, cm³										
RF	4.15	4.19	7.03	5.14	5.51	11.10	14.70	8.46	1.17	6.45
IF	5.19	4.31	3.64	6.42	3.42	3.10	7.80	4.04	1.55	4.99
RF+IF	9.34	8.50	10.67	11.56	8.93	14.20	22.50	12.50	2.72	11.44

SF indicates segmental fibrosis; RF, replacement fibrosis; and IF, interstitial fibrosis.

Discussion

The results of the present study indicate that ischemic cardiomyopathy in its terminal stage was characterized by decompensated eccentric left ventricular hypertrophy, documented by an increase in cardiac weight and a decrease in the ventricular mass-to-chamber volume ratio. Myocardial damage consisted of healed infarcts, multiple foci of replacement fibrosis across the ventricular wall, and diffuse interstitial fibrosis. These different forms of tissue injury were associated with a significant amount of myocyte loss and reactive hypertrophy of the remaining viable cells. The growth response of myocytes, however, showed an increase in cell length that exceeded the lateral expansion of the cell. Such a change in myocyte shape contributed to ventricular dilation and relative thinning of the wall. Importantly, the addition of myocyte muscle mass by cellular hypertrophy was less than that of collagen, so diffuse myocardial fibrosis and myocyte cell loss may represent the major structural rearrangements of the failing cardiomyopathic heart of ischemic origin.

Ischemic Cardiomyopathy and Ventricular Hypertrophy

Increasing pressure loading on the heart induces concentric ventricular hypertrophy in which wall thickness increases without chamber enlargement.[10,11,24] In its compensated form, mural thickening is the result of an increase in myocyte diameter with no change in the mural number of myocytes or in the aggregate number of cells in the entire ventricle.[24] The combination of these events leads to an augmentation in the ventricular

mass-to-chamber volume ratio.[10,11] On the other hand, an increased volume load typically results in eccentric ventricular hypertrophy in which chamber volume enlarges without a relative increase in wall thickness.[10,11,24] In its compensated stage, chamber dilation is produced by myocyte lengthening, whereas the ventricular number of cells is not altered.[24] Importantly, myocyte diameter also increases, so that a modest absolute increase in wall thickness occurs and the ratio of wall thickness-to-chamber radius remains constant.[24] In addition, the proportion between ventricular mass and chamber volume does not vary. When these relations are not preserved, decompensated concentric and eccentric hypertrophy develop,[10,11,24] as shown here in the form of ischemic cardiomyopathy. Specifically, the longitudinal and transverse left ventricular cavitary diameters increased 15% and 99%, respectively, provoking a 4.6-fold expansion in chamber volume. Mural thickening was not observed, and reactive hypertrophy nearly doubled the magnitude of ventricular mass inclusive of the nonmyocyte components of the myocardium. These anatomical rearrangements resulted in a marked decrease in the ratios of wall thickness-to-chamber radius and ventricular mass-to-chamber volume. Thus, eccentric left ventricular hypertrophy in its decompensated form characterized congestive heart failure of ischemic origin.

More complex is the description of the phenomena involved in the adaptation of the right ventricle in ischemic cardiomyopathy. Results showed a modest 37% expansion in wall area coupled with a 75% increase in ventricular mass. These data are consistent with an

increase in ventricular mass–to–chamber volume ratio and a concentric hypertrophic response.[10,11] However, the greater wall area implies that chamber dilation occurred under these conditions, demonstrating that anatomic indexes of cardiac decompensation were present on this side of the heart as well.

Ventricular dilation and relative wall thinning have been shown to be consistent determinants of the acute and chronic adaptations of the ventricular myocardium following ischemic injury in both humans and animal models.[9,12,16,19,25] These alterations in ventricular size and shape occur as a result of a segmental loss of muscle mass due to infarction[12-14] or as a consequence of scattered foci of tissue damage across the ventricular wall associated with severe degrees of coronary artery constriction.[26,27] Both situations encountered here most likely represent the primary factors responsible for the sequence of events that led to the increase in ventricular cavitary volume and congestive heart failure.

Two structural mechanisms recently have been identified in the genesis of ventricular dilation in ischemic cardiomyopathy. The first one involves an architectural rearrangement of the myocyte compartment with side-to-side slippage of cells within the wall and mural thinning,[15,28] whereas the second one concerns the pattern of the myocyte hypertrophic reaction in which the increase in cell length significantly exceeds the lateral growth of myocytes.[29,30] Muscle fiber slippage appears to be an early adaptive response of the wall to the elevated diastolic ventricular pressure[28,31-33] and may account for most of the acute expansions in cavitary volume associated with regional[15,28] and global[7,31-33] ischemia. However, myocyte lengthening is a chronic phenomenon that can be expected to generate a larger chamber size without affecting wall thickness.[21,29,30] Myocyte cell slippage has also been implicated in the adaptive response of the heart to pressure[34] and volume[35] overloads. In the present study, a 36% decrease in the number of myocytes included in the thickness of the left ventricular wall was found in combination with biventricular myocyte hypertrophy. In this regard, the increases in left and right ventricular myocyte length were 51% and 67%, whereas the corresponding increases in myocyte diameter were only 16% and 13%, respectively. Thus, these observations suggest that chronic myocardial ischemia is coupled with changes in the mural number of myocytes, consistent with side-to-side movement of cells within the wall, and myocyte lengthening. These anatomic variables contributed to ventricular dilation and the development of decompensated eccentric left ventricular hypertrophy. On the other hand, myocyte lengthening was the exclusive mechanism of right ventricular dilation.

Ischemic Cardiomyopathy and Myocyte Hypertrophy

Although the modifications of cardiac anatomy with ischemic cardiomyopathy demonstrated that growth compensatory mechanisms were inadequate to maintain the relation between ventricular cavitary volume and myocardial mass, these gross morphological parameters did not provide information concerning the magnitude of reactive hypertrophy in the muscle compartment of the ventricle. Changes in ventricular weight are commonly used to analyze the extent of cardiac hypertrophy associated with pathological states of the heart. This approach repeatedly has been considered to provide a reliable index of the degree of reactive growth occurring at the level of myocytes. However, this type of analysis is valid only under the conditions in which the proportion among the different structural constituents of the myocardium is not altered and the number of cells in the ventricle remains constant.[36] This is not the case after coronary occlusion and myocardial infarction or after coronary constriction and focal tissue damage. Chronic myocardial ischemia was found here to expand total myocyte mass in the left ventricle by 47%, significantly less than the 85% increase in left ventricular weight. Because of this overestimation of myocyte hypertrophy based on ventricular weight measurements, the reduction in muscle mass–to–chamber volume ratio has to be considered to be markedly greater than that previously claimed. Because chamber volume expanded 4.6-fold and the myocyte compartment 1.5-fold, a 3.1-fold deficit in muscle mass was present for a complete restoration of ventricular mass–to–chamber volume ratio in the cardiomyopathic heart. This observation was restricted to the left ventricle because the expansions in myocyte and ventricular mass were similar in the right ventricle.

The phenomenon of ischemic myocyte death, segmental and diffuse, results not only in reparative processes associated with healing and scar formation but also in a reactive hypertrophic response of the overloaded remaining cells.[6-9] Such a condition consistently has been found in the failing heart of both humans[26,30,37-39] and animal models.[40,41] Myocyte loss and muscle cell hypertrophy have been demonstrated in idiopathic dilated[42,43] and alcoholic cardiomyopathy,[44,45] aging,[7] valvular diseases,[37,39] and the presence of defects in the coronary circulation.[30] Thus, loss of cells may constitute a major determinant of myocardial dysfunction and ventricular failure in most pathological processes of the heart. In addition, the event of myocyte loss is responsible for the difference in the extent of hypertrophy detected at the organ, tissue, and cellular levels.[6,7,36] In this regard, the expansion in myocyte cell volume per nucleus obtained here markedly exceeded the increases in ventricular weight and aggregate myocyte volume after ischemic cardiomyopathy. Therefore, the characterization of muscle mass hypertrophy under this setting can be obtained only by the quantitative analysis of the changes in number and size of myocytes.[6]

Ischemic Cardiomyopathy and Myocardial Fibrosis

In recent years, several studies in humans[46] and animal models[47] have suggested that alterations of the collagen framework in the myocardium may play an important role in the genesis of ventricular dysfunction of ischemic and nonischemic origin.[47] In particular, the possibility has been advanced that collagen deposition in the cardiac interstitium may constitute a primary event leading to a depression in myocardial and ventricular pump function.[40] This contention is supported by the observations in the present study that interstitial and replacement fibrosis were found to affect the noninfarcted myocardium of both ventricles. Moreover, collagen accumulation through these two processes exceeded the quantity associated with myocardial infarction and ventricular scarring. The total amount of

connective tissue was found t comprise 28% and 13% of the left and right ventricle, respectively. In comparison with control values, these percentages corresponded to 4.5-fold and 3.4-fold increases. Because myocyte cell volume expanded by approximately 2.0- to 2.5-fold, the changes in the collagen compartment were major components of the remodeling of the ventricle with chronic ischemia.

Although there is general agreement that myocyte loss is the etiological factor of replacement fibrosis in the ventricular wall,[7,47] less clear is the mechanism responsible for activation of the cardiac interstitium, resulting in the accumulation of fibrillar collagen between myocytes. Claims have been made that hormonal and/or hemodynamic overloads[47] may trigger fibroblast proliferation and collagen neosynthesis in the myocardium independent of myocytolytic necrosis and muscle cell loss. However, death of individual myocytes occurs with coronary artery constriction,[7] and this phenomenon may stimulate discrete healing processes contributing to the expansion of the interstitium. Myocyte loss was similar in the two ventricles despite marked differences in the fractional volumes of the collagen compartment. The 2.2-fold greater volume percent of connective tissue components in the left ventricle with regard to the right suggests that collagen neosynthesis may have occurred in excess of the magnitude of myocyte loss. Alternatively, myocyte cellular hyperplasia might have been present in the left ventricle, leading to an underestimation of the extent of myocyte cell loss.

Conclusions

Scattered myocyte loss leading to the formation of multiple foci of replacement fibrosis in the myocardium, in combination with interstitial fibrosis, appears to be the major cause of ventricular remodeling in the cardiomyopathic heart of ischemic origin. Myocardial infarction is a consistent determinant of this process and contributes to the alterations in size and shape of the heart, but it does not represent the principal etiological factor in the accumulation of collagen in the ventricle with the progression of the disease. Replacement and interstitial fibrosis account for nearly 70% of the amount of fibrotic tissue in the myocardium, whereas myocardial infarction comprises approximately 30%. Importantly, these different forms of damage are only in part involved in the dilation of the ventricular chamber and the development of decompensated eccentric hypertrophy. Myocyte lengthening and slippage of cells within the wall are the predominant structural mechanisms responsible for the increase in cavitary volume under this setting. However, myocyte hypertrophy is inadequate for the preservation of the ventricular mass-to-chamber volume ratio. Thus, the ischemic cardiomyopathic process may be initiated by coronary occlusion and myocardial infarction, but its evolution is mostly controlled by a number f interrelated events occurring in the noninfarcted myocardium. How differences in treatment among patients as well as the impact of drug therapy on myocytes and collagen compartment may have influenced the remodeling f the ventricle in ischemic cardiomyopathy cannot be established at present.

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